

- (ii) otherwise obtaining a cell that expresses or will express the replication factor;
  - (b) transfecting the cell with a second vector, wherein
    - (i) the second vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker;
    - (ii) the second vector additionally contains a second DNA in operative combination with a promoter for expression of the second DNA, and which second DNA does not code for a selectable marker; and
    - (iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor, and
  - (c) expressing the second DNA, thereby obtaining a gene product encoded by the second DNA.
2. (Amended) The method according to Claim 1, wherein the replication factor is a viral replication factor.
3. (Twice Amended) The method according to Claim 2, wherein the viral replication factor is selected from polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, SV40 large T antigen.
4. (Twice Amended) The method according to Claim 1, wherein the second vector does not express the replication factor.
5. (Twice Amended) The method according to Claim 1, wherein the selectable marker is a gene product conferring antibiotic resistance.

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6. (Twice Amended) The method according to Claim 1, further comprising transfecting the cell with a third vector, wherein the third vector contains a DNA in operative combination with a promoter for expression of the DNA, and replication of the third vector is dependent upon presence within the cell of the replication factor.
7. (Amended) The method according to Claim 6, wherein the third vector expresses a selectable marker, which selectable marker is different from that expressed by the second vector.
8. (Twice Amended) The method according to Claim 1, wherein the cell is selected from the group consisting of a mammalian cell and an avian cell.

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10. (Amended) The method according to Claim 1, wherein the cell is an ES cell.
11. (Twice Amended) The method according to Claim 1, for transfection of an ES cell wherein the ES cell of step (a) expresses polyoma large T antigen and the second vector comprises a natural target for polyoma large T antigen.
12. (Twice Amended) The method according to Claim 1, wherein the second DNA codes for a polypeptide or protein.
13. (Twice Amended) The method according to Claim 1, wherein the second DNA codes for an antisense RNA.
14. (Twice Amended) The method according to Claim 1, wherein the promoter of the second vector is inducible.
15. (Twice Amended) The method according to Claim 1, wherein transcription of the second DNA can be activated by a site specific recombinase.

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16. (Twice Amended) The method according to Claim 1, wherein replication of the second vector can be prevented by a site specific recombinase.
17. (Twice Amended) A vector for transfection of a cell selected from the group consisting of an ES cell, an EC cell and an EG cell in vitro, wherein:
- (i) the vector contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker;
  - (ii) the vector contains a second DNA in operative combination with a promoter for expression of the DNA, and which second DNA does not code for a selectable marker;
  - (iii) extrachromosomal replication of the vector is dependent upon presence within the cell of a replication factor selected from the group consisting of polyoma large T antigen and papilloma virus replication factors; and
  - (iv) the vector does not express the replication factor or a fragment or portion of the replication factor.
18. (Amended) The vector according to Claim 17, wherein the vector is a viral replication factor.
20. (Twice Amended) The vector according to Claim 17, wherein the vector is free of DNA coding for the replication factor or any part thereof.
21. (Twice Amended) The vector according to Claim 17, for transfection of mammalian or avian cells.
23. (Twice Amended) The vector according to Claim 17, comprising a natural target for polyoma large T antigen.

24. (Twice Amended) The vector according to Claim 17, wherein the second DNA codes for a polypeptide or protein.
25. (Twice Amended) The vector according to Claim 17, wherein the DNA second codes for an antisense DNA.
26. (Twice Amended) The vector according to Claim 17, wherein the promoter of the second vector is inducible.
27. (Twice Amended) The vector according to Claim 17, wherein the selectable marker is a gene product conferring antibiotic resistance.
29. (Twice Amended) An ES, EC or EG cell transfected with a first vector that expresses a replication factor and with a second vector according to Claim 17, wherein the replication factor maintains the second vector extrachromosomally.
30. (Amended) The cell according to Claim 29, wherein said cell is a mammalian cell.
32. (Twice Amended) The cell selected from an ES, EC or EG cell according to Claim 29 and differentiated progeny thereof.
33. (Twice Amended) An in vitro assay for the effect of a presence in a cell, selected from the group consisting of an ES cell, an EC cell and an EG cell, of a protein or polypeptide or other product of DNA expression, comprising the steps:
- (a) (i) transfecting the cell with a first vector that expresses a replication factor; or
- (ii) otherwise obtaining a cell that expresses or will express the replication factor;
- (b) transfecting the cell with a second vector, wherein

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- (i) the second vector contains a DNA coding for the protein or polypeptide or other product of DNA expression in operative combination with a promoter for expression of the DNA;
- (ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and
- (iii) extrachromosomal replication of the second vector is dependent upon presence within the cell of the replication factor;
- (c) selecting for cells that have been transfected with the second vector;  
and
- (d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the protein or polypeptide or other product of DNA expression.
34. (Amended) The assay according to Claim 33, wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate assays in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.
35. (Twice Amended) The assay according to Claim 33, for assay of the effect of simultaneous presence in the cell of a first test factor and a second test factor, wherein said first and second test factors are independently selected from the group consisting of a protein, a polypeptide and another product of DNA expression.

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37. (Twice Amended) An in vitro method of assaying whether a DNA under investigation codes for a polypeptide that directs transport of a cell active protein to a cell surface comprising expressing in a cell selected from the group consisting of an ES cell, an EC cell and an EG cell, a composite DNA including (a) a DNA sequence under investigation, linked to (b) a DNA coding for the cell active protein, wherein
- (i) activity of the cell active protein is dependent upon transport of the cell active protein to the cell surface,
  - (ii) the DNA of (b) does not code for a polypeptide that directs transport of the cell active protein to the cell surface, and
  - (iii) the cell active protein inhibits differentiation of the cell and in the absence of the cell active protein the cell will differentiate.
38. (Amended) The method according to Claim 37, for screening a library of DNAs to identify DNA sequences coding for signal polypeptide sequences that transport proteins to the cell surface, and the method optionally comprises determining whether the cell active protein is transported to the cell surface and remains there or is secreted by the cell.
39. (Twice Amended) The method according to Claim 37, wherein the DNA of (b) is obtained by deleting or disabling, from a DNA encoding a cell surface or secreted protein, that portion of the DNA that codes for the polypeptide sequence responsible for transport of the protein to the cell surface such that the protein is not transported to the cell surface.

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40. (Twice Amended) The method according to Claim 37, wherein the cell active protein induces a morphological or proliferative change in the cell.
42. (Twice Amended) The method according to Claim 37, wherein the cell active protein is a cell surface receptor.
43. (Amended) The method according to Claim 42, wherein the cell active protein is an IL-6 receptor and the DNA of (b) encodes a modified form of the receptor preprotein lacking a functional signal sequence.
44. (Twice Amended) The method according to Claim 37, comprising investigating the properties of a DNA in mammalian or avian cells.
46. (Amended) The method according to Claim 37, comprising investigating the properties of a DNA in ES, EC or EG cells or differentiated progeny of such cells.
47. (Twice Amended) The method according to Claim 37, comprising expressing the composite DNA by:
- (a) (i) transfected the cell with a first vector that expresses a replication factor; or  
(ii) otherwise obtaining a cell that expresses or will express the replication factor;
- (b) transfected the cell with a second vector, wherein  
(i) the second vector contains the composite DNA in operative combination with a promoter for expression of the composite DNA;  
(ii) the second vector also contains a DNA coding for a selectable marker in operative combination with a promoter for expression of the selectable marker; and

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- (iii) extrachromosomal replication of the second vector is dependant upon presence within the cell of the replication factor;
- (c) selecting for cells that have been transfected with the second vector; and
- (d) maintaining the selected cells over a plurality of generations so as to assay the effect of expression of the composite DNA.
48. (Twice Amended) The method according to Claim 47, wherein step (a) is carried out once and the cells obtained are divided and used for a plurality of separate methods in which steps (b)-(d) are carried out a plurality of times with second vectors containing different DNA sequences.
49. (Twice Amended) The method according to Claim 37, for identification of a DNA coding for a cell surface or secreted protein comprising isolating the DNA under investigation.
50. (Twice Amended) The method according to Claim 37, for identification of a cell surface or secreted protein comprising isolating a protein or polypeptide encoded by the DNA under investigation.

Please add new claims 51-54 as follows:

51. (New) A composition for in vitro transfection of a cell selected from the group consisting of an ES cell, EC cell and an EG cell, said composition comprising first and at least second vectors, wherein:
- (a) said first vector comprises a DNA coding for a replication factor; and
- (b) (i) said at least second vector comprises a first DNA coding for a selectable marker in operative combination with a first promoter for expression of said selectable marker;

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- (ii) extrachromosomal replication of said at least second vector is dependent upon presence within the cell of said replication factor; and
  - (iii) said at least second vector does not express a replication factor or a fragment or portion thereof.
52. (New) The composition of Claim 51 wherein the viral replication factor is selected from the group consisting of polyoma large T antigen, EBNA-1 antigen, papilloma virus replication factors, and SV40 large T antigen.
53. (New) The composition of claim 51 wherein the selectable marker is a gene product conferring antibiotic resistance.
54. (New) A cell comprising first and at least second vectors, wherein:
- (a) said first vector comprises a DNA coding for a replication factor; and
  - (b) (i) extrachromosomal replication of said at least second vector is dependent upon presence within the cell of said replication factor; and
  - (ii) said at least second vector does not express a replication factor or a fragment or portion thereof.

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